AMENDMENTS TO THE CLAIMS

Please amend claims 1-5, 7-29, 36-46. Please cancel claim 35. Deletions appear in ~~strikethrough font~~, and additions are underlined. This listing of claims will replace all prior versions and listings of claims in the application:

Complete listing of claims

1. (Currently amended) A polymer affinity matrix ~~for binding one or more substances in a fluid for removing said substance(s) from the fluid and/or decreasing the amount or concentration thereof in said fluid with a view to preventing, eliminating, or reducing undesired activation of components or processes in said fluid, wherein said matrix comprises~~ comprising

a) a solid support

b) at least one spacer bound to the solid support, and, coupled to each spacer,

c) ~~a~~ at least one ligand containing at least one binding unit having at least one functional group,

wherein the polymer affinity matrix has the ability to selectively bind to at least one substance in a fluid. ~~said substance(s).~~

2. (Currently amended) The polymer affinity matrix according to claim 1, wherein said at least one ligand has a defined three-dimensional structure which is complementary as regards charge and/or hydrophobicity/hydrophilicity to the three-dimensional structure of a binding motif of said at least one ~~one or more~~ substance. ~~(s),~~

3. (Currently amended) The polymer affinity matrix according to ~~any one of~~ claims 1 ~~and 2~~, wherein the at least one ligand is represented with the formula

$-X^1{}_n-Y_m[X^2{}_i-Z^1; X^3{}_j-Z^2]_{½(m+1)}$, (general Formula I),

wherein

n = 0 or 1;

$m = 2^k - 1$;

k = 0 to 10, wherein if k = 0 then $X_2 = X_3$ and $Z_1 = Z_2$;

i = 0 or 1; and

j = 0 or 1,

or

$-(X^1{}_n-Y^1[Y^2{}_m[X^2{}_i-Z^1; X^3{}_j-Z^2]_{½(m+1)})_r-X^4{}_p-Z^3$, (general Formula II),

wherein

n = 0 or 1;

$m = 2^k-1$;

k = 0-10, wherein if k = 0 then $X_2 = X_3$ and $Z_1 = Z_2$;

r = 1-100;

i = 0 or 1;

j = 0 or 1; and

p = 0 or 1;

wherein Z^1, Z^2 and Z^3 each independently of each other represents the at least one binding unit and ~~is each~~ each is an organic molecule chosen from ~~the group con-~~

~~sisting of an~~ amino acids, ~~a~~ peptides, ~~a~~ fatty acids, ~~a~~ carbohydrates, ~~a~~ lectin, and ~~a~~ nucleotides, and derivatives thereof, ~~or~~ and combinations thereof,

wherein Y, Y^1 and Y^2 each is independently of each other a trifunctional branching molecule chosen from ~~the group consisting of~~ amino, hydroxy, aldehyde, isocyanate, isotiocyanate, thiol, maleimido, and epoxy, and derivatives thereof, ~~or~~ and combinations thereof, and

wherein X^1, X^2, and X^3 each, is independently of each other, an optional bifunctional distance molecule containing two functional groups chosen from ~~the group consisting of~~ amino, carboxy, hydroxy, aldehyde, isocyanate, isothiocyanate, thiol, maleimido, and epoxy, and derivatives thereof, ~~and/or~~ and combinations thereof;

wherein optionally the ligand is cyclic.

~~or wherein the ligand is cyclic and wherein the ligand is cyclic~~.

4. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding claims~~ claim 1, wherein the at least one ligand comprises 1 to 100 functional groups.~~, preferably 1-32 functional groups~~.

5. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claims ~~1~~, wherein the at least one ~~each~~ binding unit is an amino acid, at least a part of which is positively charged at about physiological pH of blood.

6. (Original) The polymer affinity matrix according to claim 5, wherein the amino acid has a pK_A of ≥ 6.0.

7. (Currently amended) The polymer affinity matrix according to claim 6, wherein the amino acid is arginine, lysine, histidine, or cysteine~~, preferably arginine~~.

8. (Currently amended) The polymer affinity matrix according to claim 7, wherein the amount or concentration of the amino acid is 0.01 to 5 mmol/g matrix.

9. (Currently amended) The polymer affinity matrix according to claim 8, wherein the amount or concentration of the amino acid is chosen from ~~is about~~ 0.01, 0.1, 1, 2, 3, 4 ~~or~~ and 5 mmol/g matrix.

10. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claims ~~5~~, wherein the number of amino acid molecules per ligand is chosen from 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 ~~or~~and 16~~, preferably 4-8~~.

11. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claims ~~5~~, wherein the amino acid is arginine and the ~~amount or~~ concentration of arginine is ≤ 3 mmol/g matrix.

12. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claims ~~1~~, wherein said at least one functional group is chosen from ~~an~~ amino group or substituted amino group, a carboxy group, a hydroxy group, a thiol group, a

guanidino group, ~~or~~ and combinations thereof ~~preferably an amine group or guanidino group~~.

13. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claims 1, wherein the at least one ligand has a tree- or comb-like structure chosen from: ~~as shown in the following formulas:~~

~~Examples of ligands having the general Formula I, i.e., $-X^1_n-Y_m[X^2_i-Z^1;X^3_j-Z^2]_{1/2(m+1)}$, wherein it here is~~

~~$-Lys_m[Arg]_{(m+1)}$, i.e.,~~

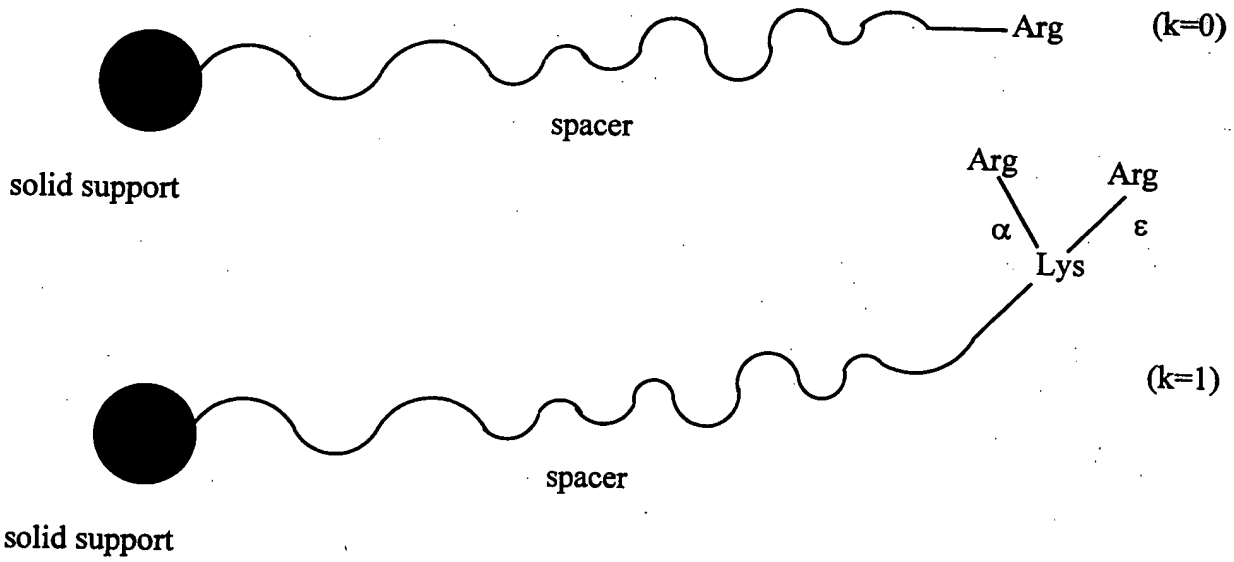
~~$n = l = j = 0$;~~

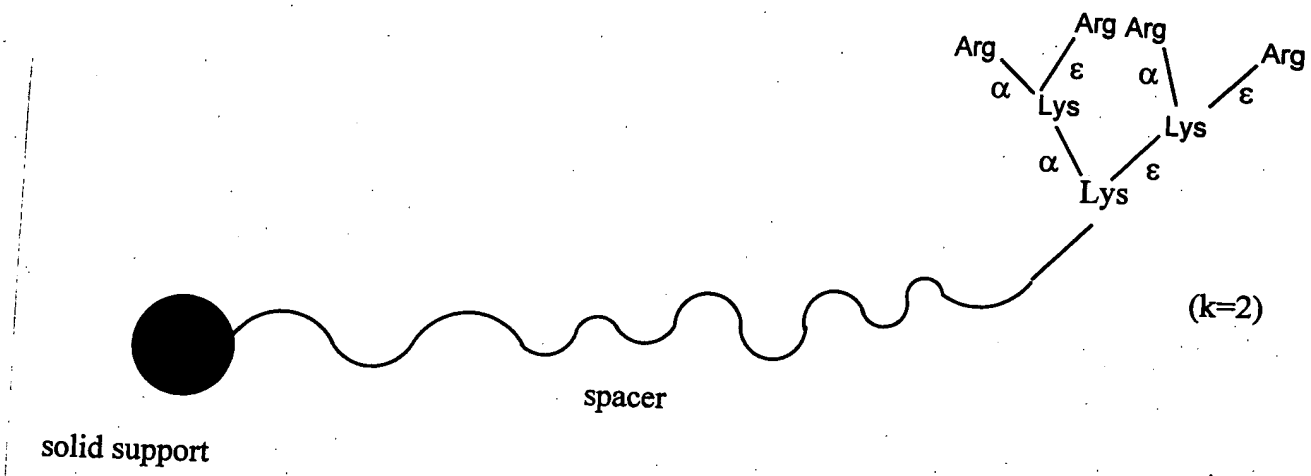
~~$m = 2^k - 1$~~

~~$z^1 = Z^2 = Arg$;~~

~~all Y = Lys; and~~

~~X^1, X^2, and X^3 are absent:~~




Arg
Arg
Arg
Arg
Arg
α
ε
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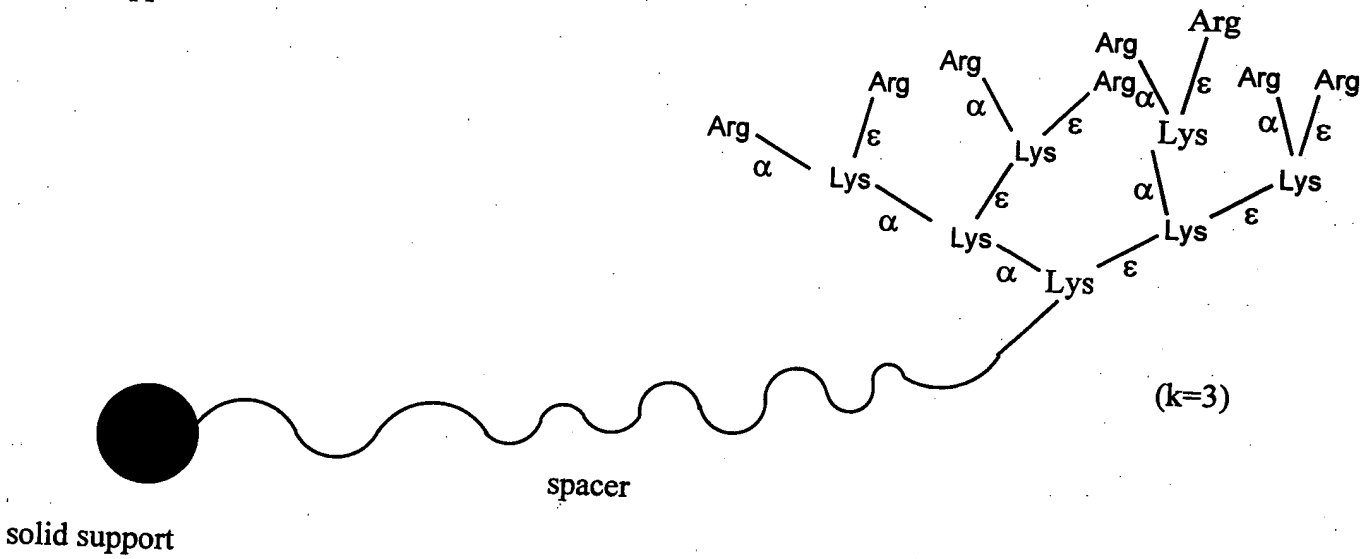
ε
Lys
Lys
α
ε
Lys
(k=2)
spacer
solid support


Arg
Arg
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Arg
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α
ε
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α
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α
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Lys
Lys
Lys
Lys
α
ε
α
ε
Lys
Lys
α
ε
Lys
(k=3)
spacer
solid support

~~Examples of ligands having the general Formula II, i.e.~~

~~$-(X^1)_n-Y^1-[Y^2)_l-Z^1;X^3)_j-Z^2]_{1+(m+1)})_r-X^4)_p-Z^3$, wherein it here is~~

~~$-(Lys[Lys_m^{[Arg]}(m+1)\,r^{-H}$, i.e.~~

~~$n = l = j = p = o;$~~

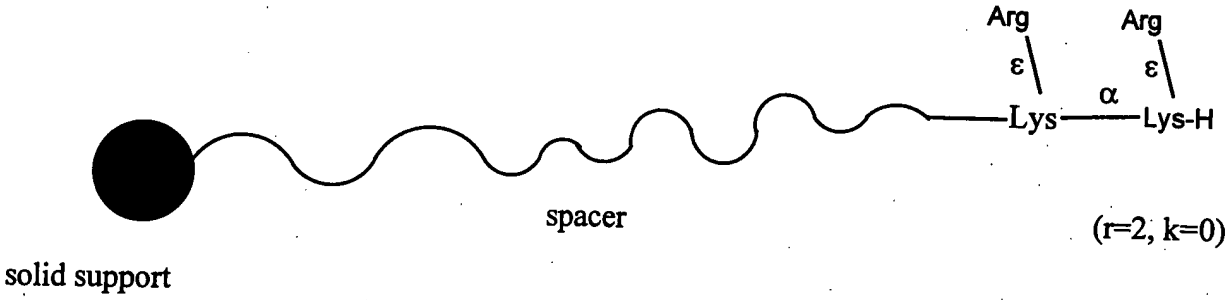
~~$m = 2^k-1$~~

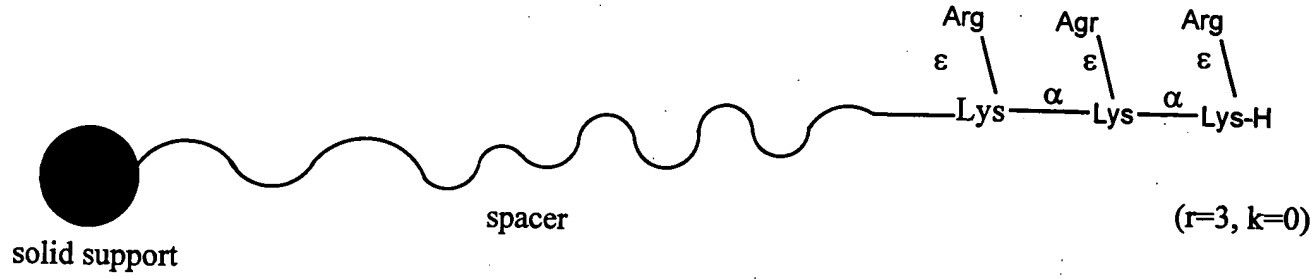
~~$z^1 = z^2 = Arg;$~~

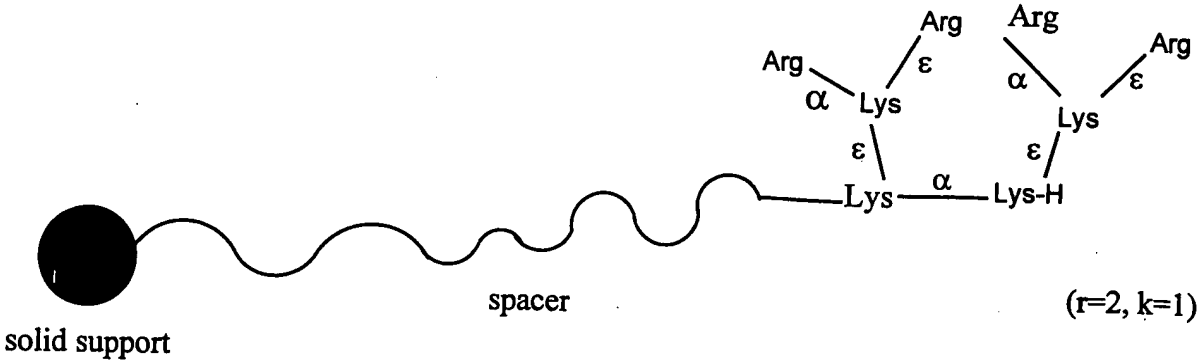
~~$z^3 = H;$~~

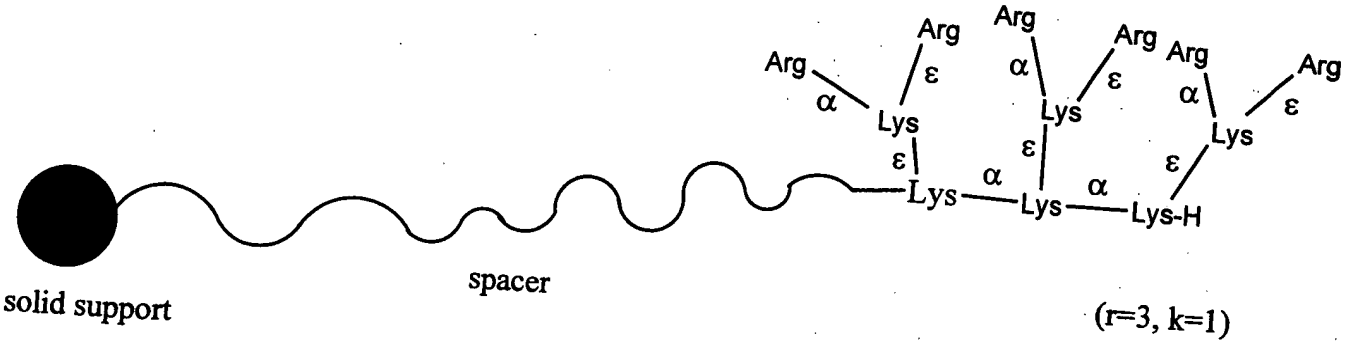
~~all Y = Lys;~~

~~X^1, X^2, X^3 and X^4 are absent:~~

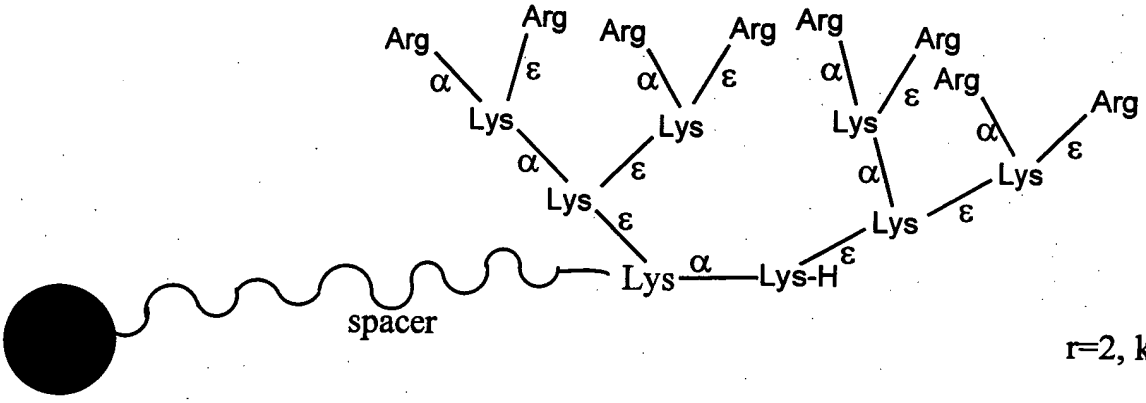




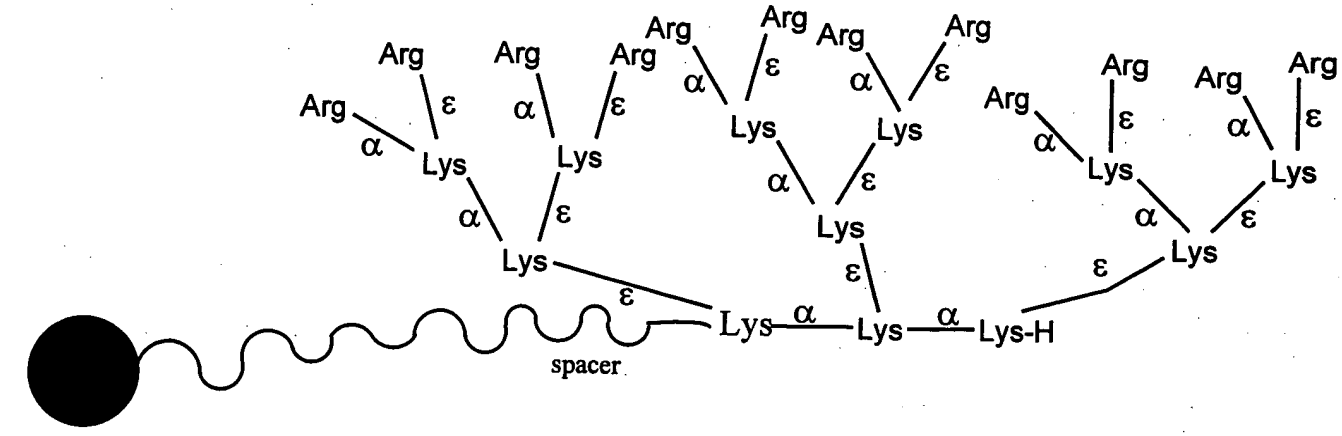




(r=3, k=1)

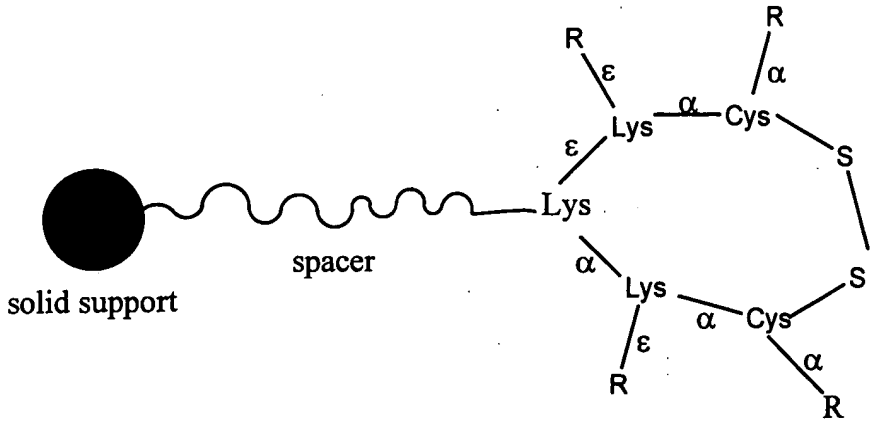


r=2, k=2



(r=3, k=2)

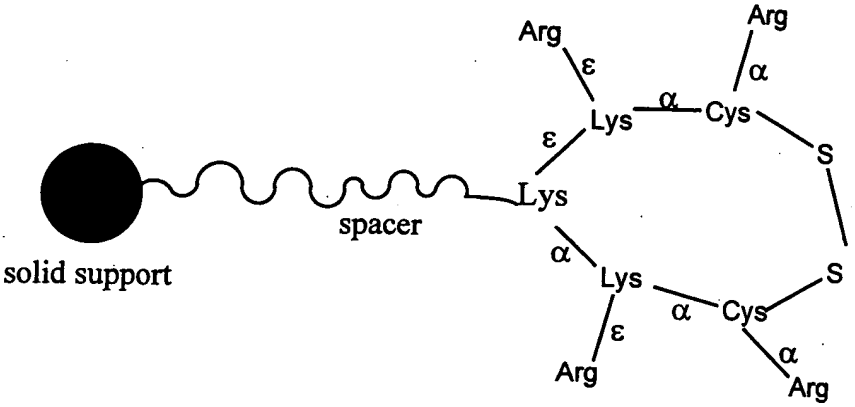
~~and/or includes a cyctic structure, preferably as shown in the following formulas:~~



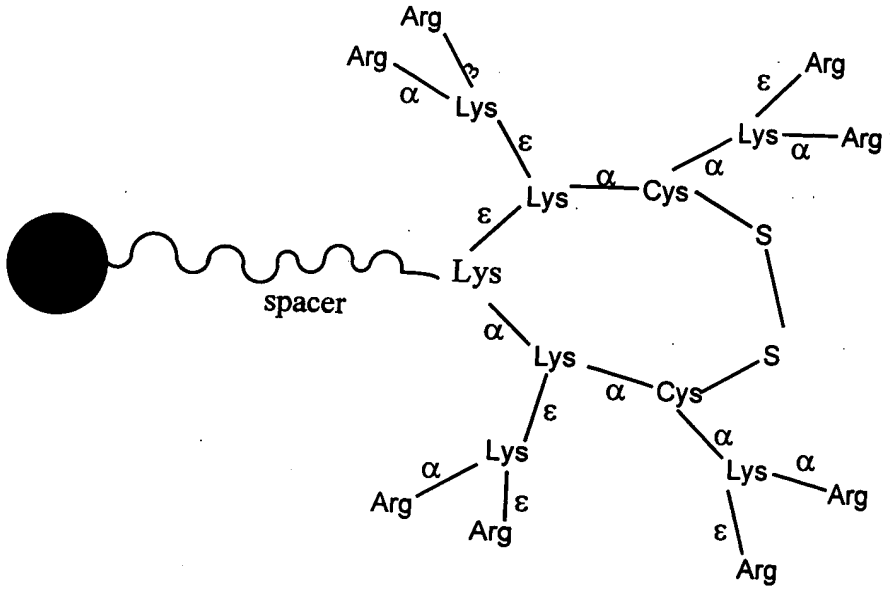
$R= - Lys_m[Arg]_{(m+1)}$

$m=2^k-1$

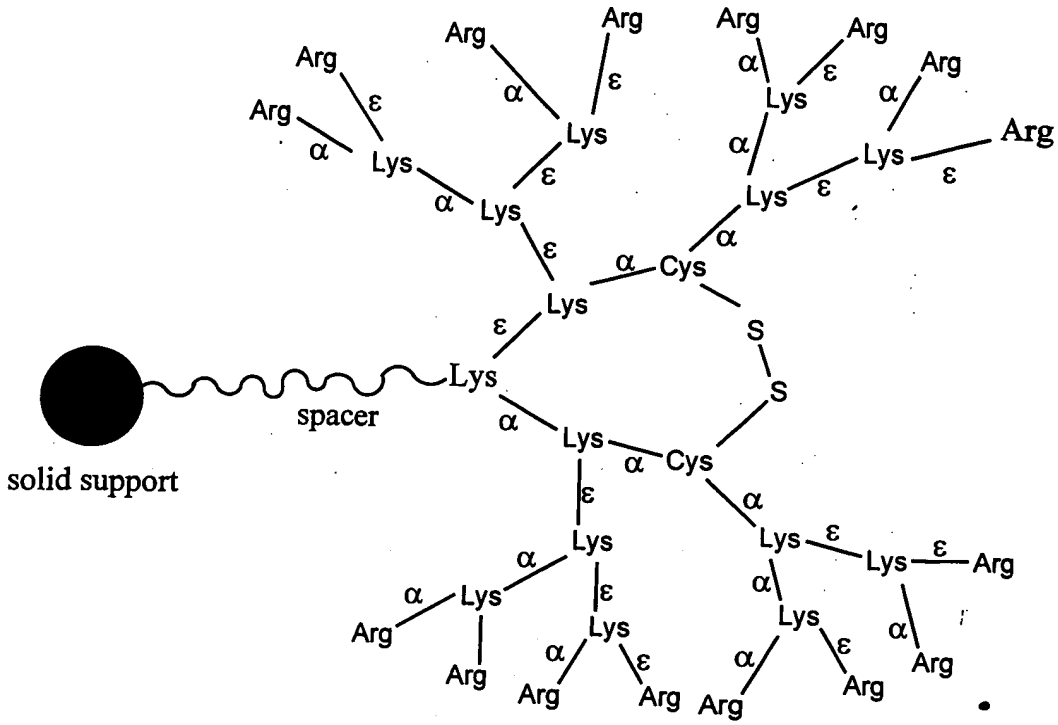
(k = 0)



(k=1)



(k=2)



14. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claims ~~1~~, wherein positive charges of at least two of the at least one functional groups are separated from each other by a distance defined by the distance between individually negatively charged groups in the binding motif of the at least one substance ~~(s) to be bound , preferably phosphate groups in an endotoxin~~.

15. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claims ~~1~~, wherein the at least one spacer is substantially hydrophobic or hydrophilic and has the function of an anchoring part for the at least one ligand.

16. (Currently amended) The polymer affinity matrix according to claim 15, wherein the at least one spacer is ~~selected from the group consisting of~~ chosen from poly- or oligoethylene glycols of the formula $H\text{-}(OCH_2CH_2)_n\text{-}OH$, wherein n represents 2 to 250, ~~or~~ polyvinylalcohols, polyvinylamines, polyolycidoles, polyethyleneimines, and polypropyleneoxides, ~~or~~ and derivatives thereof.

17. (Currently amended) The polymer affinity matrix according to claim 16, wherein the at least one spacer is chosen from ~~a~~ polyethylene glycol (PEG) in a linear and/or branched configuration and ~~has~~ having an average molecular weight of 400 to 10,000 Daltons, ~~or~~ and derivatives thereof.

18. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claims ~~1~~, wherein the solid support is made of a material ~~selected~~ chosen from ~~the group consisting of~~ polystyrene, polyvinyl alcohols, polyhydroxystyrenes, polymers produced from chloromethylated polystyrenes or polyacrylates, polymethacrylates functionalised with hydroxy groups, hydroxyalkyl-polystyrenes, hydroxyaryl-polystyrenes, hydroxyalkyl-aryl-polystyrenes, polyhydroxyalkylated polystyrenes, polyhydroxyarylated polystyrenes, isocyanatoalkyl-polystyrenes, isocyanatoaryl-polystyrenes, carboxyalkyl-polystyrenes, carboxyaryl-polystyrenes,

aminoalkyl-polystyrenes, aminoaryl-polystyrenes, polymethacrylates, cross-linked polyethyleneglycols, cellulose, silica, carbohydrates, latex, cyclo-olefine copolymers, and glass ~~or~~ and combinations thereof.

19. (Currently amended) The polymer affinity matrix according to claim 18, wherein the solid support has the form of a bead, gel, membrane, particle, net, woven or non-woven fabric, fibre mat, tube, film, foil or combinations thereof or cross-linked interpenetrating networks~~, preferably a cross-linked polystyrene~~.

20. (Currently amended) The polymer matrix according to ~~any one of the preceding~~ claims ~~1~~, wherein said polymer matrix is biocompatible and has a swelling capacity enough to allow perfusion of whole blood.

21. (Currently amended) The polymer matrix according to claim 20, wherein the swelling capacity is about 1.5 to 20 fold~~, preferably 2-6 fold,~~ from a dry state to the hydrated form.

22. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claims ~~1~~, wherein said polymer matrix provides a three-dimensional complementary structure for binding the at least one substance chosen from ~~selected from the group consisting of~~ bacteria or virus derived constituents~~,~~ endotoxins~~,~~ exotoxins~~,~~ bacterial DNA ~~or~~ and fragments thereof~~,~~ oligonucleotides; cells~~, in particular endothelial cells, stem cells, and tumour cells~~; blood cells~~, in particular lymphocytes,~~

~~thrombocytes, granulocytes, dendritic cells, and monocytes;~~ prions~~;~~ parasites~~;~~ fungi~~;~~ drugs after overdosing~~;~~ pathogenic food additives~~;~~ products from acute or chronic metabolic disturbances resulting from diabetes mellitus, liver disease, uraemia, kidney diseases or inflammation~~;~~ heparin~~;~~ bacteria and viruses~~;~~ pathogen-loaded blood cells~~,~~ or at least parts or degradation products thereof~~;~~ DNA~~;~~ phosphate~~;~~ cytokines~~;~~ growth factors~~;~~ hormones~~;~~ chemokines~~;~~ uremic toxins~~;~~ blood clotting proteins~~;~~ procoagulatory proteins~~;~~ inflammatory or proinflammatory proteins~~;~~ macrophage migration inhibitory factor~~;~~ soluble or cell surface bound proteins~~;~~ soluble adhesion molecules~~;~~ ~~and~~ glucose or degradation products thereof~~;~~ pyrogens~~;~~ bacterial exotoxins~~;~~ and products from Gram-positive bacteria~~, preferably lipoteichonic acid, in particular bacterial pyrogene, preferably endotexins, in particular the lipid A component of lipopolysacchjarides (LPS)~~.

23. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claim~~s~~ 1, wherein said polymer matrix has a cut-off value ranging ~~of~~ from ~~about~~ 1 x 10^2 to ~~about~~ 1 x 10^6 Daltons and binds hydrophobic and/or hydrophilic substances or hydrophobic and hydrophilic substances.

24. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claim~~s~~ 1, wherein the fluid ~~which the substance(s) is/are to be removed from or to be reduced in~~ is an aqueous or organic solution~~;~~ a body fluid~~;~~ preferably blood; therapeutical fluids~~;~~ fluids for life science applications~~;~~ ~~preferably buffer solutions~~ infusion fluids or dialysis fluids in biological, diagnostic or biotechnological applications~~;~~

blood products obtained from healthy donors;~~, such as plasma, platelet concentrates, erythrocyte concentrates which are used for transfusions, blood substitutes, preferably oxygen carriers, modified hemoglobin solutions and artificial hemoglobin solutions;~~ fluids for nutrition; and fluids for industrial use.

25. (Currently amended) The polymer affinity matrix according to ~~any one of the preceding~~ claims ~~1~~, wherein the solid support is a cross-linked polystyrene, the at least one spacer is a polyethylene glycol and the at least one each binding unit is arginine.

26. (Currently amended) A method for removing one or more substances from a fluid and/or reducing the amount or concentration thereof ~~with a view to preventing, eliminating or reducing undesired activation of components or processes in said fluid,~~ comprising contacting the fluid with the polymer affinity matrix of claim 1 ~~as defined in any one of claims 1~~ for a period of time sufficient to reduce the amount or concentration or remove said at least one substance~~(s)~~.

27. (Currently amended) The method according to claim 26, wherein the period of time ~~is~~ranges from 1 to 2 hours.

28. (Currently amended) The method according to ~~any one of~~ claim 26~~and 27~~, wherein the at least one substance is an endotoxin and the fluid is blood, wherein the amount or concentration of endotoxin after being removed or reduced is below the

capacity of activating components in blood or prevents activation of components or processes in blood.

29. (Currently amended) ~~The~~ A method for producing a polymer affinity matrix as defined in ~~any one of~~ claims ~~1-25~~, comprising

a) attaching the spacer to the solid support

to obtain a first complex, and

b) attaching to said first complex the ligand containing said at least one binding unit with at least one functional group; or

c) attaching the spacer to the ligand containing said at least one binding unit with at least one functional group to obtain a second complex, and

d) attaching the solid support to said second complex;

or

e) attaching the spacer to the solid support to obtain a first complex, and

f) solid phase synthesis of the ligand on the spacer bound to the solid support, or

g) building up or synthesizing the spacer from monomers directly on the solid support by grafting, and

h) attaching to said first complex the ligand containing said at least one binding unit with at least one functional group, or

i) building up or synthesizing the spacer from monomers directly on the solid support by grafting, and

k) solid phase synthesis of the ligand on the spacer bound to the solid support;~~,~~

wherein information about ~~the~~ three-dimensional structure, presence of charges and hydrophobic/hydrophilic regions of the binding motif on the substance~~(s)~~ to bind is collected from X-ray crystallography, protein sequencing, protein modelling or hydrophobicity and hydrophilicity calculations and the ligand containing the binding unit is made complementary as regards charge and/or hydrophilicity/hydrophobicity to the binding motif of said substance(s).

30. (Original) The method according to claim 29 comprising the steps of,

for a) and b), activation of the solid support, coupling of the spacer molecule on the solid support, synthesis of the ligand containing the binding unit, and site specific coupling of the ligand to the spacer molecule, or,

for c) and d), synthesis of the ligand containing the binding unit, coupling of the spacer molecule to the ligand, activation of the solid support, and site specific coupling of the spacer-ligand complex to the solid support, or,

for e) and f), activation of the solid support, coupling of the spacer molecule to the activated solid support, and solid phase synthesis of the ligand on the spacer bound to the support.

31. (Original) The method according to claim 29 comprising the steps of,

for a) and b), activation of the spacer, coupling of the activated spacer to the solid support, and coupling the ligand to said activated spacer, or,

for c) and d), synthesis of the ligand, activation of the spacer, site specific coupling of the ligand to the activated spacer molecule and coupling of the spacer-ligand complex to the solid support, or,

for e) and f), activation of the spacer, coupling of the activated spacer to the solid support and solid synthesis of the ligand on the spacer bound to the solid support.

32. (Currently Amended) The method according to claim 26, wherein the ~~Use of the polymer affinity matrix as defined in claims 1 for removal of one or more substances, from a fluid, or decreasing the amount or concentration thereof in said fluid, preferably a body~~ fluid ~~or a therapeutic fluid, most preferably~~ is blood or serum.

33. (Currently amended) A The method ~~Use~~ according to claim 32 wherein the method results in ~~for~~ production of less activated blood or prevention of undesired activation of components or processes in blood.

34. (Currently amended) The method ~~Use~~ according to claim 33, wherein the method is ~~as a~~ part of an extracorporeal blood purification process or ~~as~~ is used in an implant in the body to contact blood or any body fluid. ~~, preferably in the vascular system, blood vessels or the peritoneal cavity.~~

35. (Cancel) ~~Use according to claim 34 for production of less activated blood or prevention of undesired activation of components or processes in blood.~~

36. (Currently amended) A kit for removing one or more substances from a fluid or decreasing the amount and/or concentration thereof in said fluid ~~with a view to preventing, eliminating, or reducing undesired activation of components or processes in said fluid, said kit~~ comprising a polymer affinity matrix as defined in ~~any one of~~ claims 1-~~25~~.

37. (Currently amended) The kit according to claim 36, wherein it further comprises sample tubes, and a device for extra- and/or intracorporeal treatment of said fluid.~~, preferably blood or serum~~.

38. (Currently amended) A method for producing ~~Use of a polymer matrix for the production of~~ a polymer affinity matrix for removal of one or more substances from a fluid or decreasing the amount or concentration thereof in said fluid.

wherein the specific affinity of the polymer affinity matrix is dependent on any ligand applied on the polymer matrix,

wherein the polymer matrix includes a solid support and at least one ~~a~~ spacer, wherein the solid support is made of a material ~~selected~~ chosen from ~~the group consisting of~~ polystyrene, polyvinyl alcohols, polyhydroxystyrenes, polymers produced from chloromethylated polystyrenes or polyacrylates, polymethacrylates functionalised with hydroxy groups, hydroxyalkyl-polystyrenes, hydroxyaryl-polystyrenes, hydroxyalkyl-aryl-polystyrenes, polyhydroxyalkylated polystyrenes, polyhydroxyarylated polystyrenes, isocyanatoalkyl-polystyrenes, isocyanatoaryl-polystyrenes, carboxyalkyl-polystyrenes, carboxyaryl-

polystyrenes, aminoalkyl-polystyrenes, aminoaryl-polystyrenes, polymethacrylates, cross-linked polyethyleneglycols, cellulose, silica, carbohydrates, latex, cyclo-olefine copolymers, and glass ~~or~~ and combinations thereof, preferably a cross-linked polystyrene, and

wherein the at least one spacer is ~~selected~~ chosen from ~~the group consisting of~~ poly- or oligoethylene glycols of the formula $H-(OCH_2CH_2)_n-OH$, wherein n represents 2-250.

39. (Currently amended) The method ~~Use~~ according to claim 38, wherein the solid support has the form of a bead, gel, membrane, particle, net, woven or non-woven fabric, fibre mat, tube, film, foil or combinations thereof or cross-linked interpenetrating networks.

40. (Currently amended) The method ~~Use~~ according to claim 38, wherein the at least one spacer is chosen from ~~a~~ polyethylene glycol (PEG) in a linear and/or branched configuration and has an average molecular weight of 400-10 000 Daltons, ~~or~~ and derivatives thereof.

41. (Currently amended) The method ~~Use~~ according to ~~any one of~~ claims 38-~~40~~, wherein the polymer matrix has a swelling capacity enough to allow perfusion of plasma or whole blood.

42. (Currently amended) The method ~~Use~~ according to claim 41, wherein the swelling capacity is about 1.5 to 20 fold~~, preferably 2-6 fold~~, from a dry state to the hydrated form.

43. (Currently amended) The method ~~Use~~ according to ~~any one of~~ claims 38-~~42~~, wherein the polymer matrix has the form of gel type beads.

44. (Currently amended) The method ~~Use~~ according to ~~any one of~~ claims 38-~~43~~, wherein said fluid is an aqueous or organic solution~~;~~, a body fluid~~;~~ ~~preferably blood,~~ therapeutic fluids~~;~~, fluids for life science applications~~;~~ ~~preferably buffer solutions,~~ infusion fluids or dialysis fluids in biological, diagnostic or biotechnological application~~;~~, blood products obtained from healthy donors~~;~~ ~~such as plasma, platelet concentrates, erythrocyte concentrates, preferably oxygen carriers, modified hemoglobin solutions and artificial hemoglobulin solutions,~~ fluids for nutrition; and fluids for industrial use.

45. (Currently amended) The method ~~Use~~ according to ~~any one of~~ claims 38-~~44~~, wherein said polymer matrix has a cut-off value ranging ~~of~~ from ~~about~~ 1 x 10^2 to ~~about~~ 1 x 10^6 Daltons and binds hydrophobic and hydrophilic substances or hydrophobic and/or hydrophilic substances~~. or hydrophobic or hydrophilic substances.~~

46. (Currently amended) The method ~~Use~~ according to ~~any one of~~ claims 38-~~45~~, wherein the solid support is a cross-linked polystyrene, and the at least one spacer is a polyethylene glycol.